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Flow-Related Mechanics of the Intervertebral Disc: The Validity of an *In Vitro* Model

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Study Design. An *in vitro* mechanical study on porcine motion segments.

Objectives. To test the validity of *in vitro* studies of the flow-related mechanics of the intervertebral disc and, in particular, to investigate whether fluid flows back into the disc during unloading after a loading cycle.

Summary of Background Data. *In vivo* studies show both the inflow and outflow of fluid in the intervertebral disc. The resistance to flow out of the disc is higher than to inflow. The fluid flow is regulated *via* unbalance between the external load and the osmotic pressure of the nucleus pulposus.

Materials. There were 8 porcine lumbar motion segments (without posterior elements) and 8 isolated discs tested in a physiologic saline bath (39°C). The specimens were preloaded at 0.025 MPa for 15 minutes. Three 15-minute loading periods at 2.0 MPa were applied, each followed by an unloading period of 30 minutes. Loads, axial displacements, and nucleus pressure were recorded online.

Results. Over the 3 loading and unloading periods, all specimens showed a net loss of height and mass. The time series of specimen height during the 3 unloading periods showed virtually identical responses. The pressure in the nucleus decreased in the subsequent loading periods and showed no increase during unloading.

Conclusion. The data show the limitations of an *in vitro* model for studying fluid flow-related intervertebral disc mechanics. During loading, outflow of fluid occurred, but inflow appears to be virtually absent during unloading. Poro-elastic behavior cannot be reproduced in an *in vitro* model.

Key words: intervertebral disc, biomechanics, fluid flow, testing. **Spine 2005;30:E534–E539**

The mechanical behavior of a spinal motion segment is highly nonlinear.[1,2] To a large extent, this result is attributable to the complex composition of the intervertebral disc, which shows both viscoelastic and poro-elastic behavior.[3] The intervertebral disc and, in particular, the nucleus pulposus have a high water content. The hydration of the disc varies under the influence of loading. Consequently, fluid flow plays an important role in the mechanical behavior of the intervertebral disc.[3–9] In a healthy disc there is a continuous tendency toward equilibrium between the external load and swelling pressure of the disc.[10] The swelling pressure is dependent on the proteoglycan concentration in the nucleus. Thus, the swelling pressure depends on the degree of hydration of the nucleus. When the external load changes, the proteoglycans will imbibe or express water until the swelling pressure balances the external load and a new equilibrium is reached.

After a night's rest, daily activity causes an increase in intradiscal pressure due to gravity and, in particular, to muscle forces.[11,12] As a result, fluid is expressed from the nucleus, which will increase the proteoglycan concentration and swelling pressure, until equilibrium is reached.[13,14] During resting periods, the flow direction is reversed and fluid flows back into the intervertebral disc,[15] and the disc regains its properties. The main path for fluid flow from the disc supposedly leads through the endplate into the vertebral body.[16] The vertebral body and the intervertebral disc are connected *via* channels through the bony endplate.[17] Recently, it has been suggested that the resistance of these channels to flow is direction-dependent, with the resistance to flow of fluid into the intervertebral disc being lower than the resistance to outflow.[18] This effect allows full recovery of fluid content during night rest.

Our knowledge of the mechanical behavior of the intervertebral disc is largely based on *in vitro* testing of cadaveric material.[19,20] Yet, it is not clear whether mechanical behavior of the disc *in vitro*, caused by fluid inflow and outflow, resembles *in vivo* behavior. The results of several studies raise doubts as to whether this is the case, especially for fluid inflow, which is apparently reduced *in vitro*.[21–23] In this study, we investigated the mechanical behavior of intervertebral discs *in vitro*. The mechanical behavior of the intervertebral disc, under alternating axial compression and relaxation, was assessed in intervertebral discs taken from the lumbar spine of pigs. Intact motion segments as well as isolated intervertebral discs were tested.

Fluid flow, to and from the disc, plays an important role in the mechanical behavior of the disc. Flow cannot be measured directly in intact motion segments. Changes of specimen height, changes of pressure in the nucleus,

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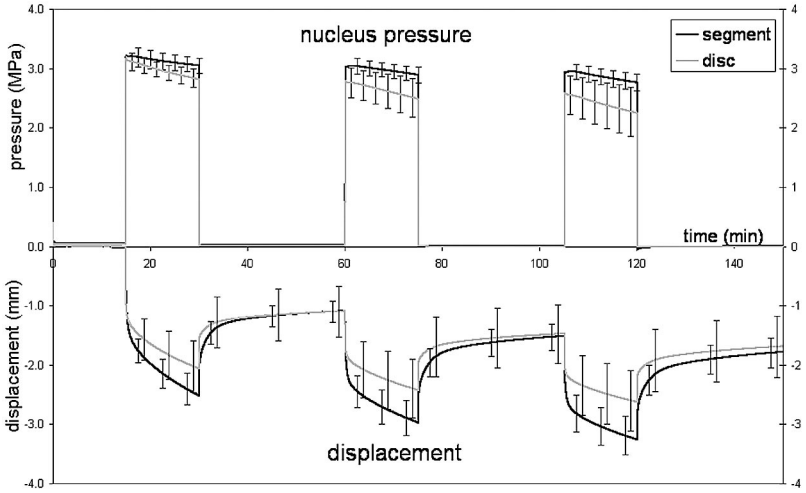

Figure 1. Changes in average nucleus pressure and specimen height.

and loss of mass of the intervertebral discs were used to monitor changes in mechanical behavior under alternating loads.

■ Materials and Methods

The lumbar spines (L1–L5) of 8, 10-month-old pigs, were harvested and frozen for later usage. One spinal motion segment, comprising 2 vertebrae and an intervertebral disc, and 1 one isolated intervertebral disc, including its adjacent endplates, were taken from each lumbar spine. The motion segment was L2–L3 in one half of the cases and L3–L4 in the remaining cases. The associated isolated discs were L4–L5 and L1–L2, respectively. The posterior part of the motion segment was removed at the pedicles to exclude interference with the facet joints. To obtain isolated disc specimens, the adjacent vertebral bodies were cut off as close to the endplate as possible. Subsequently, the cutting edge was brushed clean. The specimens were thawed before testing. Each isolated intervertebral disc was weighed (Mettler-Toledo, Greifensee, Switzerland) before and after testing to estimate differences in fluid content. The change of mass of the complete motion segments could not be measured because of the embedding method of the motion segments.

In the testing device, an Instron material testing machine (Instron 8872, Canton, MA), the isolated disc specimens were placed between 2 porous plates. During axial compression, the porous plates allowed free passage of fluid to and from the endplates of the disc. The top plate was attached to the testing device *via* a ball-and-socket joint. However, the complete motion segments had to be embedded in aluminum cups for stability. The outer endplates of the specimen were embedded into the cups in bismuth. The cups were attached to the material testing machine. The posterior part of the motion segment was removed at the pedicles. Thus, an open connection for fluid into and from the vertebral body was created. All tests were performed in a bath with physiologic saline at a temperature of 39°C, which is the body temperature of pigs.

The total area of the intervertebral disc was measured. This area was then used to calculate the required force to obtain an overall pressure load of 2.0 MPa. The forces corresponded to approximately 2 times the body weight of the animals. The specimens were preloaded at 0.025 MPa (20 N) for 15 minutes. Subsequently, they were loaded for 3 full loading cycles, each consisting of a loading period of 15 minutes at 2.0 MPa and an unloading period of 30 minutes at 0.025 MPa. The nucleus pressure was measured with a pressure needle (Gaeltec LTD, Dunvegan, Scotland), which was inserted into the nucleus and remained in position during the whole test. The load, displacement, and pressure in the intervertebral disc were recorded at a frequency of 2 Hz.

■ Results

The average area of the specimens was 848 mm^2, thus, average external compression load was 1694 N. Over the 3 loading and unloading periods, all specimens showed a net loss of height (Figure 1). The total loss of segment height was 1.76 mm ± 0.23 mm and of isolated discs was 1.58 ± 0.47 mm. The loss of height after the complete loading protocol shows that a total of 90 minutes unloading with virtually no compression load did not compensate for the loss of height as a result of the 45 minutes of loading. The loss of height in whole segments was larger than that in isolated discs in each separate cycle of loading and unloading (Table 1). The reduction in height after each load change is compared between cycles in Figures 2, 3. Overlaying the time series of the specimen height during loading show that the loss of height decreased after each load cycle, while the responses in the unloading period were virtually identical. The slope of the displacement curves during loading of the successive load cycles, which was calculated over the interval from 5 to 15 minutes, decreased significantly ($P < 0.01$) over the successive loading cycles. Although the slope of the displacement curve during unloading, calculated over the same interval as during the loading

Table 1. Specimen Height Change After Each Complete Cycle*

	Segment (mm)	Intervertebral Disc (mm)
Cycle 1	−1.07 ± 0.16	−1.01 ± 0.38
Cycle 2	−0.42 ± 0.06	−0.36 ± 0.08
Cycle 3	−0.27 ± 0.05	−0.21 ± 0.04

*Loading plus unloading.

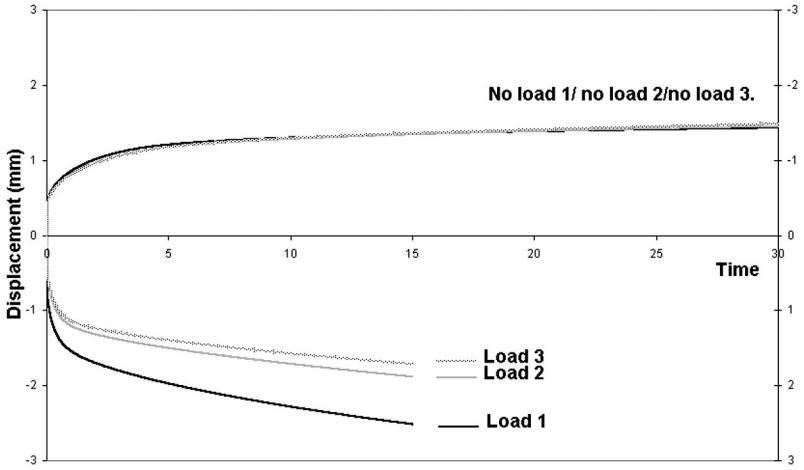

Figure 2. Overlaying height change data of 3 successive loading and unloading periods of the motion segments.

curve, increased in all specimens (Figure 4), this effect was marginal, and the responses in the subsequent unloading periods have virtually identical appearances. The ratios of the slope during loading/unloading in the successive cycles were 8.6, 5.1, and 3.8 for the isolated discs, and 8.9, 4.8, and 3.5 for the motion segments increased.

In all 3 loading periods, the pressure of the nucleus decreased more or less linearly. This effect was more pronounced in the isolated discs than in the motion segments. The rate of nucleus pressure reduction in the isolated discs was approximately twice as high as the rate of pressure reduction in the motion segments (Figure 1, Table 2). During the relaxation phase, the nucleus pressure did not change significantly, and the pressure showed no sign of an increase during unloading. At the onset of the next loading cycle, the nucleus pressure had not recovered. All isolated intervertebral discs showed a significant reduction in mass after testing ($P < 0.03$). The average mass of the discs before the test was 14.72 ± 0.52 g, and the average loss of mass after 3 complete load cycles was 0.65 ± 0.19 g.

Discussion

It is generally assumed that the external load and osmotic pressure control the fluid content of the nucleus.[9,20] The intervertebral disc tends continuously, *via* attracting and expressing water from the nucleus, toward equilibrium between the external load and swelling pressure. *In vivo* measurements of the intradiscal pressure in human discs have shown a gain in nucleus pressure during rest.[11] *In vivo*, an unloading period of approximately 7 of 24 hours is sufficient for recovery of the disc's water content[15] and disc height.[24,25] In other words, the fluid balance in the intervertebral disc is shifted toward outflow of fluid during the day and reverts after removal of the external load in favor of fluid inflow during the night. Therefore, fluid flow is an important aspect in disc mechanics.

In the current research, we have investigated whether the *in vivo* mechanical behavior can be reproduced *in vitro*. After a repeated axial compression loading and unloading cycle, all specimens showed a net loss of height and mass in an *in vitro* test. Specimen height showed virtually identical responses during the 3 unloading periods. The pressure in the nucleus decreased in the subsequent loading periods and showed no increase during unloading.

Hydration of the disc depends on the loading history of the disc.[10] The swelling pressure is not a material property of the nucleus tissue, but it changes with hydra-

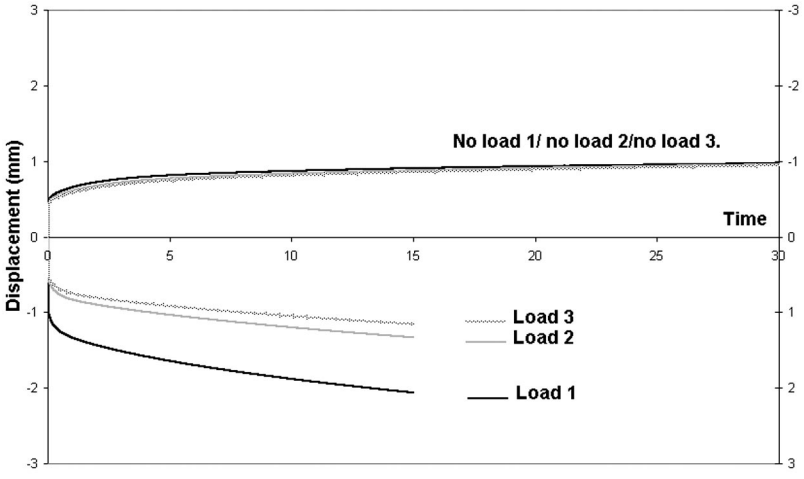

Figure 3. Overlaying height change data of 3 successive loading and unloading periods of the isolated discs.

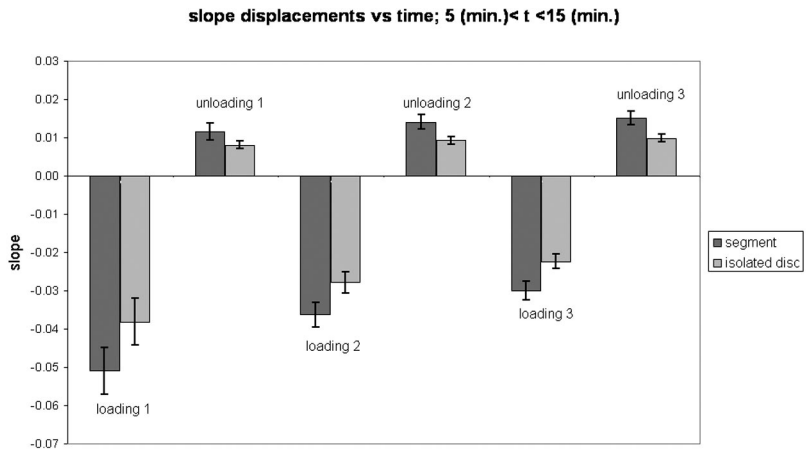

Figure 4. Average slope of the displacement curves in the interval from 5 to 15 minutes of the successive loading and unloading cycles.

tion of the disc. It is possible that the specimens at testing were not equally hydrated. However, because test specimens have not been tested immediately postmortem, it is likely that at the moment of testing, the specimens had sufficient time to adapt to the new unloaded situation. In the tests, this effect was confirmed in the results of the preloading phase when the load was close to zero (20 N compression load for 15 minutes). No major changes in specimen height or pressure were seen during this test phase.

The compression tests have been performed on a porcine specimen. Although the biochemical composition of the porcine intervertebral disc differs from the human intervertebral disc, the porcine disc provides, with respect to functional characteristics and anatomic characteristics, an accepted model.[26,27] Furthermore, in pilot studies we have seen similar effects as described here in different species (*e.g.*, goat, human being). Therefore, the porcine disc appears to be a valid model for this aspect of disc mechanics.[28]

The applied compression load corresponds to 2 times the body weight of the animal. In comparison with the static load in human beings, the test load can be considered high. However, based on the compression strength of the vertebral body of quadrupeds, the *in vivo* compression load in quadrupeds is expected to be higher than in human beings.[29] The main reason is that the major part of the spinal load is related to muscle forces, which are likely to be higher in quadrupeds than in human beings.

Table 2. Rate of Average Nucleus Pressure Reduction (MPa/min)

	Segment	Disc
Loading phase 1		
Slope	−0.011 ± 0.014	−0.022 ± 0.004
R^2	0.99	0.99
Loading phase 2		
Slope	−0.011 ± 0.012	−0.020 ± 0.004
R^2	0.98	1.00
Loading phase 3		
Slope	−0.014 ± 0.011	−0.023 ± 0.003
R^2	0.98	1.00

The test environment of the discs was as close as possible to the physiologic, *in vivo*, environment. The temperature was increased to the body temperature of the animal. This process will influence decomposition of the disc; the decomposition rate will be increased compared to a test at room temperature. Because the temperature was kept constant during testing, the influence on the decomposition rate could be studied by comparing the successive loading and unloading phases. Overlaying the time series of the displacements of the successive unloading periods showed virtually identical responses (Figures 2, 3). Therefore, it is unlikely that the changes in displacement curves between successive loading cycles can be attributed to decomposition, while nothing is seen in successive unloading curves. This result is confirmed by tests on ovine intervertebral discs[30] and by pilot tests we performed for this research. These pilot tests were performed on porcine segments wrapped in saline-soaked gauze at room temperature, and showed a similar absence of recovery of disc height and intradiscal pressure.

For practical reasons we have chosen to use frozen materials. Although it has been argued in one study that this could influence fluid flow in the disc,[31] another more recent study from the same group revealed no major effects of careful frozen storage over a period comparable to the one used in the present study.[32] In addition, we have repeated the experiment on a single fresh specimen to see if the freezing and thawing of the discs had affected the results. The same absence of recovery was observed in this fresh specimen. The test protocol in the current study was load controlled. During every loading phase, the load was kept constant. Nevertheless, the measured nucleus pressure decreased in all specimens during the loading phase. Because the sum of all loads is constant by definition, this can only be explained if the load is internally redistributed from a load-bearing nucleus to a load-bearing anulus fibrosis. This result corresponds with earlier findings in the literature.[6,8] During the unloading

phase, the pressure in the nucleus did not change significantly. The nucleus pressure showed no sign of an increase at the start of the next loading phase. The load did not shift back from the anulus to the nucleus. Therefore, the load-bearing capacity of the nucleus was not restored.

The difference between the motion segments and the isolated discs was the presence of the vertebral body. In the isolated discs, the rate of pressure reduction in the nucleus was twice as high as compared to the motion segments (Figure 1, Table 1). Thus, the removal of the vertebral body resulted in a faster reduction in nucleus pressure. In comparison, the reduction in specimen height of the motion segment was larger than the loss of height of the isolated disc. This effect appears to conflict with the larger pressure reduction in the isolated discs. However, the change of height of the motion segment is a combined reduction in disc and vertebral body height. Considering the stiffness of the vertebral bodies, the data suggest that the removal of the vertebral bodies has reduced the resistance against fluid outflow.

Overlaying the time series of the displacements of the successive unloading periods, the displacements showed virtually identical responses. The loss of mass indicated that fluid was expressed from the nucleus. The specimen height gain did not increase after each loading cycle, while the pressure gradient increased as a result of fluid loss. This effect suggests that during the unloading phase, the mechanical responses were not determined by the osmotic pressure gradient. Therefore, we hypothesize that the height gain after loading was dominated by viscoelasticity. Because fluid flow cannot be measured directly, we have to rely on indirect measurements for an explanation. Recovery time was twice as long as the loading time, thus, it was expected that the disc would regain its original properties. However, the continuous loss of nucleus pressure, specimen height, and, especially, loss of mass in the current study show the opposite. In this test, all indicators point toward the absence of fluid flow into the disc. This leads to the conclusion that the disc and, in particular, the nucleus did not regain their fluid content during unloading. The discharged fluid remained outside the disc. The permanent loss of fluid was confirmed by the statistically significant loss of mass in the isolated discs. This result raises the question as to why the nucleus did not regain its fluid content during the unloading phase. One reason could be that proteoglycans could be dissolved[33,34] in the expressed fluid. This result would directly lead to lower proteoglycan content in the next load step and a shift in the equilibrium toward a reduced drive to the inflow of fluid into the disc.

We hypothesize that the fluid flow is hampered, *in vitro*, by congestion of the small pores in the endplate that function as primary pathways for fluid flow. The pores in the endplate might be blocked from one side. The pressure of the fluid, because of the external load, could be able to push the clots, which block the pores, away from the discharge opening and transport the fluid beyond the endplate. In that case, the channels remain closed for a reversed flow direction along a much less steep pressure gradient. It is conceivable that during inflow, the clots are pushed into the endplate pores, thus creating effectively a one-way valve. This result would explain why the pressure reduction in isolated discs was steeper. With the vertebral body removed, the endplate is more accessible for fluid outflow, while the pores in the endplate remain closed for inflow.

The outcome of these experiments is consistent with the results of other studies in which no or limited recovery was found.[22,23] In one study, it has been reported that disc mechanics are restored after recovery.[21] However, the unloading phase during this study was 6 times longer than the loading phase. Duration of the unloading phase was 18 hours, suggesting that inflow was slow compared to the outflow.[35] The intervertebral disc is a very complex structure. Its mechanical behavior comprises viscoelastic, poro-elastic, and biochemical aspects. Testing of the disc is likewise complex. The findings of the present study point at limitations of mechanical testing *in vitro*, especially when fluid inflow is vital to the mechanical behavior of the disc. Apparently mechanical behavior of the intervertebral disc under alternating loads cannot be validly studied in spinal motion segments or isolated intervertebral discs *ex vivo*.

■ Key Points

- A mechanical study *in vitro* was performed on porcine intervertebral discs under alternating loads.
- The purpose was to test the validity of *in vitro* studies of the flow-related mechanics of the intervertebral disc.
- All specimens showed a net loss of height, loss of intradiscal pressure, and mass.
- Recovery of the intervertebral disc after mechanical loading cannot be validly studied *in vitro*.

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